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Targeted and non-targeted forensic profiling of black powder substitutes and gunshot residue using gradient ion chromatography – high resolution mass spectrometry (IC-HRMS)

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Abstract

A novel and simplified gradient IC-HRMS approach is presented in this work for forensic profiling of ionic energetic material residues, including low-order explosives and gunshot residue (GSR). This new method incorporated ethanolic eluents to facilitate direct coupling of IC and HRMS without auxiliary post-column infusion pumps that are traditionally used to assist with gas phase transfer. Ethanolic eluents also enabled better integration with an in-service protocol for direct analysis of high-order organic explosives by IC-HRMS, without requiring solvent exchange before injection. Excellent method performance was achieved, enabling both full scan qualitative and quantitative analysis, as required. In particular, linearity for 19 targeted compounds yielded $R^2 > 0.99$ across several orders of magnitude, with trace analysis possible at the low-mid pg level. Reproducibility and mass accuracies were also excellent, with peak area %RSDs < 10 %, t_R %RSDs < 0.4% and $\delta_{m/z}$ < 3 ppm. The method was applied to targeted analysis of latent fingermarks and swabbed hand sweat samples to determine contact with a black-powder substitute containing nitrate, benzoate and perchlorate. When combined with principal component analysis (PCA), the effect of time since handling on recorded signals could be interpreted further in order to support forensic investigations. In a second, non-targeted application, PCA using full scan IC-HRMS data enabled classification of GSR from three different types of ammunition. An additional 20 markers of GSR were tentatively identified *in silico*, in addition to the 15 anions detected during targeted analysis. This new approach therefore streamlines and adds consistency and flexibility to forensic analysis of ionic energetic material. Furthermore, it also has implications for targeted, non-targeted and suspect screening applications in other fields by expanding the separation space to low molecular weight inorganic and organic anions.

Keywords: forensic science, explosive analysis, ion chromatography, high resolution mass spectrometry, fingermarks, non-targeted analysis

1. Introduction

Recent high-profile terrorism-related incidents have highlighted the frequency of homemade or improvised explosive devices (IEDs) in planned attacks. For the sake of illustration, the bombing events in Madrid (2004), London (2005), Bali (2002 and 2005), Oslo (2011), Boston (2013), Paris (2015), Brussels (2016) and Manchester (2017) alone claimed the lives of 745 people and injured more than 4,700. Consequently, the rapid and reliable identification of ingredients used in IEDs, as well as the potential to trace them back to specific terrorist cells/people, are currently topics of considerable interest in both forensic and security-based investigations [1, 2].

With the high accessibility of ingredients on the legal market and the ease of synthesis, IEDs often contain energetic mixtures of different inorganic salts and/or organic compounds [1]. Typical examples are ANFO (ammonium nitrate and fuel oil), black powders (nitrate salts, sulfur, charcoal), chlorate/- or perchlorate/sugar devices, as well as smokeless powders [3, 4]. These particular explosives usually leave a characteristic trace after manipulation or detonation and are composed of organic and inorganic ions, amongst other compounds [5]. A particular post-blast tracer of energetic materials is gunshot residue (GSR), which may also contain similar substances and has a particular composition that can be characteristic of the manufacturer [6]. Many analytical approaches are used for detection of these ions, at both bulk and trace levels [7, 8]. These often involve orthogonal analysis with capillary electrophoresis (CE) or ion chromatography (IC), the latter of which has become popular for routine application due to its enhanced robustness [9]. Coupling with suppressed conductivity detection (SCD) [3, 10-15] or direct/indirect UV photometric detection (UVD) [16, 17] has become common, but these detectors offer low selectivity in forensic applications, especially in terms of structural information. Furthermore, both selectivity and quantification is more challenging in complex environmental or biological matri-

ces, reducing the usefulness of the data generated for the investigation of time intervals or the comprehensive profiling of traces.

Recently, mass spectrometry (MS) has attracted interest as a more useful detection method for ionic energetic material residues, even if its adoption has generally been slow in forensic science in comparison to other fields [18-20]. In particular, high resolution mass spectrometry (HRMS) is promising for coupling to IC, as it enables fast measurements of wide *m/z* ranges with very high resolving powers (up to 140,000 FWHM for Orbitrap-based analysers) and mass accuracy (< 5 ppm). These features allow full scan data acquisition with minimised interference from isobaric ions [21, 22], which are particularly common at the low *m/z* ranges often observed in low-order explosives analysis. Other benefits include improvements in method selectivity, quantification capabilities in complex matrices and increased flexibility with data analysis, by allowing retrospective, non-targeted analysis or suspect screening to aid the *in silico* identification of new species [23, 24].

One of the main challenges of coupling IC and MS for the analysis of ionic explosive residues, however, lies in the use of high proportions of water in eluents that can lead to two specific issues pertinent to forensic analysis. Firstly, they do not enable efficient gas-phase transfer during electrospray ionisation (ESI) [25]. Secondly, their compatibility with traditional organic solvents used for sample extraction is limited, since most sampling protocols involve the use of organic solvents to ensure recovery of both the inorganic and organic compounds. Usually, these two problems are addressed by infusing organic solvents in the eluate using an auxiliary pump prior to ESI and solvent exchange with water before injection [26, 27]. The full procedure is nonetheless lengthy and complicated, and often results in sub-optimal sensitivity. With the increased solvent compatibility of modern IC resins, the direct use of organic-solvent containing eluents (i.e., acetonitrile or methanol) has been suggested as a solution to these problems; an approach that has been referred to as “solvent-enhanced IC” [18, 28]. No published method, however, has specifically been de-

signed to fit with solvents actually used in forensic extraction/sampling strategies for energetic materials, which are usually based on ethanol, isopropanol or their 50:50 mixtures with water [29-31]. As a consequence, published IC-HRMS (and generally IC-MS) methods in this field are still very limited. New developments are necessary in order to progress the science and align better with reversed-phase liquid chromatography-based analysis workflows that also frequently use HRMS detection [32].

Thus, the aim of this work was to develop and validate a new gradient IC-HRMS method that was fully compatible with current extraction/sampling methodologies, and also simultaneously added simplicity and knowledge to current approaches to ionic energetic materials residue analysis. A ratio of 50:50 ethanol:water was specifically evaluated for implementation as an IC eluent because of its extensive use as an extraction/sampling solvent for explosives-related material in the UK [31]. Firstly, gradient conditions were optimised and assessed for 19 anions of interest, which has not been demonstrated using such an IC-HRMS arrangement before. Optimal IC-HRMS (Orbitrap) coupling was then performed via a design-of-experiments (DOE) approach and the analytical performance assessed. Finally, the method was tested for energetic materials analysis in two example applications: (a) pre-blast residues of a black-powder substitute (Pyrodex) in palm sweat and fingermarks; and (b) GSR (i.e., post-discharge residues of smokeless powders). Both targeted and non-targeted data analyses were performed to highlight the potential added value of the application of full scan IC-HRMS to profiling. This is the first time that retrospective analysis of IC-HRMS data has been applied to detect new chemical components that may be useful for energetic material classification purposes.

2. Experimental

2.1. Chemicals and materials

Water was of Milli-Q grade with a specific resistance of 18.2 MΩ cm^{-1} and obtained from a Synergy UV ultra-purification system (Millipore Corp., Bedford, USA). Methanol (MeOH), ethanol (EtOH), and isopropanol (iPrOH) were obtained from Fisher Scientific (Loughborough, UK). Acetonitrile (MeCN) was obtained from Sigma-Aldrich (Gillingham, UK). All the solvents were of HPLC grade.

A total of 25 anions commonly encountered in the analysis of ionic explosive residues were selected. All were used for the optimisation of the chromatographic step, but only 19 were included in the final IC-HRMS method as the remaining six ions fell below the lower *m/z* threshold of the HRMS instrument. In addition, stable isotope-labelled nitrate ($^{15}NO_3^-$) was used as an internal standard (IS) during method validation and application, mainly to take into account small IC retention time shifts. The list of all target anions is given in Table 1. Standard reference materials were obtained from different manufacturers, as reported in Table S1 in the electronic supplementary material (ESM). Specifically, chloride, chlorite, chlorate, perchlorate, cyanate, nitrite, nitrate, azide, thiocyanate, sulfate, thiosulfate, phosphate, oxalate, threonate, phthalate and benzoate were selected because of their previous association with explosive compounds or related degradation products and, thus, forensic relevance [5]. Fluoride, acetate, bromide, bromate, chromate, formate, tartrate, lactate and citrate were added because they commonly occur in environmental samples or sweat and could thus be used for interference control and qualitative purposes, as well as to improve classification in profiling applications. Carbonate was excluded because it was used as eluting ion species.

Individual stock solutions were prepared at 1000 mg L^{-1} in Milli-Q water and stored in the dark at 4 ℃ in a refrigerator. Stocks were re-prepared monthly. Working solutions were freshly prepared daily by further dilution of stock solutions, using an appropriate solvent that matched the composition of the eluent (unless otherwise specified).

Analytical grade (98 %) sulfuric acid served as IC suppressor regenerant and was obtained from VWR Chemicals (Lutterworth, UK). Carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) were used as eluting ion species and were obtained from sodium carbonate and sodium bicarbonate. Sodium carbonate was purchased from Sigma-Aldrich and sodium bicarbonate from BDH Chemicals Ltd. (Poole, UK). Both were of analytical grade (≥ 99.5 %). Filters used for particle removal from sample extracts were syringe-driven PES membrane 0.22 µm filters from Jet Biofil (Madrid, Spain).

2.2. IC instrumentation and conditions

All separations were performed on a gradient 850 Professional IC equipped with an 858 Professional Sample Processor (Metrohm AG, Herisau, Switzerland). This instrument was fitted with an SCD detector Metrohm IC Professional Detector, an ion trap Metrohm Metrosep A Trap 1 (100 x 4 mm, 5.0 µm particle size) providing eluent purification before the injection valve, and a 3-step cartridge suppressor (Metrohm MSM Rotor A). The latter was chemically regenerated on-line, using 100 mM H_2SO_4, by a peristaltic pump at 0.8 mL min^{-1}. Separations were performed on a Metrohm Metrosep A Supp 5 column (250 x 2 mm, 5.0 µm particle size).

Eluent conditions were optimised by injection of mixed solutions of target anions at 10 mg L^{-1}. The optimised IC gradient used two eluent reservoirs composed of CO_3^{2-}/HCO_3^- in 50:50 EtOH:H_2O at concentrations of 1/0.3 mM (eluent A) and 15/4.5 mM (eluent B), programmed according to the following profile: 0 % B at 0 min, hold at 0 % B for 4.5 min, linear ramp to 100 % B for 30 min, hold at 100 % B for 3.5 min, return to 0 % B using a step gradient. Re-equilibration time was 23 min. Column temperature was kept at 50 ℃ throughout.

For comparison, the developed IC-HRMS method was compared to a more traditional aqueous-based IC-HRMS method that was optimised using the same target anions.

This involved the use of two eluent reservoirs composed of CO_3^{2-}/HCO_3^- in pure water at concentrations of 2/0.6 mM (eluent A) and 15/4.5 mM (eluent B), programmed as follows: 0 % B at 0 min, hold at 0 % B for 6.5 min, linear ramp to 100 % B for 20 min, hold at 100 % B for 24.5 min, return to 0 % B using a step gradient. Finally, re-equilibration at initial conditions was allowed for 19 min before the next run. Column temperature was kept at 40 ℃ throughout.

The injection loop in all cases was 20 µL and the eluent flow rate was 0.18 mL min^{-1} for both methods. Solvents used for preparation of mixed standard solutions and suppressor regenerant matched the eluent (i.e., EtOH:H_2O or pure water). Instrument control and data processing were performed on MagIC Net software version 3.1 from Metrohm.

2.3. HRMS instrumentation and conditions

The IC instrument was coupled directly to an Exactive Orbitrap (Thermo Fisher Scientific, Sunnyvale, USA) without any additional post-suppressor solvent infusion following the SCD, thus providing dual detection in a single run. The HRMS was equipped with a heated ESI source (Thermo HESI-II) operated in negative ionisation mode. Full scan mode was used to acquire data over a 50 – 500 *m/z* range. Instrument resolution was set to the maximum value of 140,000 FWHM (at *m/z* 200).

HRMS conditions were optimised using a design-of-experiments (DOE) approach. Optimisation was split into two independent steps (see details in ESM). Firstly, the capillary, tube lens and skimmer voltages were optimised by direct infusion of a mixed solution of seven selected probes (i.e., nitrate, perchlorate, benzoate, threonate, sulfate, oxalate and citrate) at a concentration of 10 mg L^{-1}. Following this, the IC was directly coupled to HRMS and capillary temperature, HESI-II heater temperature, sheath gas flow and spray voltage were further optimised by injection of a mixed 1 mg L^{-1} standard of the same seven probes.

The final optimised conditions were as follows: capillary voltage: –40 V; tube lens voltage: –60 V; skimmer voltage: –20 V; capillary temperature: 300 ℃; HESI-II heater temperature: 350 ℃; sheath gas flow: 25 arbitrary flow units; auxiliary gas flow: 10 arbitrary flow units; spray voltage: –4 kV. All data acquisition was performed using XCalibur software version 2.2 (Thermo).

2.4. Performance evaluation

Selectivity, precision, linear range, limits of detection (LODs) and limits of quantification (LOQs) were assessed for the fully optimised method through injection of mixed solutions of the 25 target anions. As little/no detector noise existed using HRMS in many cases, ICH guidelines were followed [33]. Repeatability was measured as the relative standard deviation (%RSD) of chromatographic retention times and peak areas, determined by the successive analysis of replicate samples (n = 6) spiked at two concentrations of 100 μg L^{-1} and 1000 μg L^{-1}. Determination ranges were studied on solutions spiked at 9 different concentrations between 1 and 5000 μg L^{-1} and were analysed in duplicate. Both linear and quadratic calibration models were applied to data points and lines of best fit giving coefficients of determination (R^2) ≥ 0.99 over the largest range were accepted. Finally, LODs and LOQs were estimated from linear calibration curves of the different anions and were respectively defined as 3 and 10 times the estimated standard deviations of peak areas divided by the slope.

2.5. IC-HRMS analysis of Pyrodex residues in sweat samples

Palm sweat and fingermarks were obtained by asking a volunteer to handle 2 g of a Pyrodex powder for about 2 minutes. Pyrodex P powder (FFFg equivalent) was purchased from Hodgdon (Vista Drive, Kansas, USA). In particular, the volunteer was asked to rigorously wash their hands twice before powder handling (firstly with soap and normal tap wa-

ter, then just with tap water to remove soap residues), as well as to clap their hands multiple times after handling to dislodge any loose particles. The powder itself was initially analysed by extracting 500 mg in 20 mL of 50:50 EtOH:H_2O for 24 h under ambient conditions with agitation. The extract was passed through a 0.22 µm filter to remove suspended particles, diluted 100-fold and analysed with IC-HRMS.

Palm sweat samples were collected from the volunteer's right hand by swabbing with cotton balls using a protocol analogous to the standard operating procedures employed at the Forensic Explosives Laboratory (Dstl, UK) and similar to that of Song-Im *et al.* [34, 35]. Swabs were prepared by soaking in 5 mL of 50:50 EtOH:H_2O contained in 27 mL glass bottles. Immediately before sampling, swabs were removed from their bottle, excess solvent squeezed out with cleaned plastic forceps and then used to swab the palm. Swabs were then transferred to empty 20 mL plastic syringe barrels and back-extracted using again 50:50 EtOH:H_2O (Terumo, Bagshot, UK). The plungers were used to firmly squeeze out the extracts into different glass tubes. After this, the plungers were removed and a further 5 mL of 50:50 EtOH:H_2O were allowed to gravimetrically pass through the compressed swabs for about 10 min. These were finally compressed with the plungers again to expel any remaining extract. Mixtures of EtOH:H_2O have been extensively used within medical and anti-bacterial wipes, showing their safe use on surfaces and human skin [36]. 50:50 EtOH:H_2O was thus considered lowly hazardous for this application.

Experiments were repeated at incremental times since initial handling of Pyrodex, i.e. t = 0, 1 and 3 h. During this time, the volunteer was asked to continue with their normal daily activities without washing their hands. A second series of experiments was performed after asking the volunteer to wash their hands with normal tap water immediately after Pyrodex handling. For this series of experiments, swab sampling was performed only twice since handling, i.e. t = 0 and 1 h. All extracts were passed through 0.22 µm filters, spiked at 1 ppm with the IS and injected onto the IC system in triplicate. Extracts obtained

without washing hands after handling Pyrodex were additionally diluted 10-fold before spiking. Negative control samples were obtained by sampling volunteer's hands before Pyrodex handling.

Fingermarks were made by depositing the thumb, index and middle fingers of the volunteer's left hand on separate circular 15 mm glass microscope coverslips (Thermo Fisher Scientific, Braunschweig, Germany). These were collected at t = 0 and 1 h after Pyrodex handling. Microscope slides were then placed in individual and sealable 10 mL glass bottles for subsequent extraction using 1 mL of 50:50 EtOH:H_2O for 24 h (ambient conditions, with agitation). All extracts were passed through 0.22 µm filters, spiked at 1 ppm with the IS and injected directly onto the IC-HRMS system in triplicate. Negative control fingermarks were obtained before Pyrodex handling. Written informed consent was obtained from the volunteer and this research was conducted with King's BDM Research Ethics Subcommittee approval (Refs: HR-15/16-1962 for fingermarks and HR-17/18-4078 for hand swabs).

2.6. IC-HRMS analysis of gunshot residue (GSR)

GSR was collected directly from spent casings from three different manufacturers of 9 mm Parabellum ammunition: Remington (Madison, North Carolina, USA), Federal (Anoka, Minnesota, USA) and Geco (Thun, Switzerland). Spent cases were donated by the Metropolitan Police Service (UK) after having been fired with the same 9 mm pistol. These were placed into individual sealable plastic bags and kept in the dark at 4 ℃ until extraction. Extraction of GSRs was carried out in line with a previously developed method [37]. Briefly, each spent casing was placed into a 10 mL glass bottle, filled with 900 µL of 50:50 EtOH:H_2O and ultrasonicated for 30 min. The extract solutions were then filtered using 0.22 µm filters, spiked at 1 ppm with the IS and injected onto IC-HRMS in triplicate. Negative control samples were obtained by analysis of the pure solvent.

2.7. Data analysis

Acquired data was initially screened for the 19 targeted anions. The most intense ion for each of them was selected and its isotope pattern recorded. Preliminary identifications were accepted if the retention time deviation was < 5 % of error compared to reference standards and if accurate *m/z* was < 5 ppm [38]. Relative isotopic abundances (RIAs) were then measured for all the detectable isotopes (or at least one) and compared to theoretical values. Following the suggestions of Knolhoff *et al.* [39], identities were thus accepted if absolute RIA deviations were: < 5 % if the intensity of the monoisotopic peak was > 10^7 counts, < 10 % if the monoisotopic peak was between 10^6 and 10^7 counts, or < 15 % if the monoisotopic peak was < 10^6 counts.

Non-targeted analysis was performed on GSR samples in order to detect new chemical components that may aid classification. Peak picking was done through MZmine 2 software. All signals of > 10^5 counts were shortlisted to generate a list of accurate *m/z* values. This list was used to build a set of extracted ion chromatograms (EICs), which were deconvoluted using a local minimum search feature. Chromatographic peaks were then detected and aligned between the different data files. This procedure produced a final list of potentially useful *m/z* signals. Those corresponding to the 19 targeted compounds were removed at first to avoid duplication. The list was then manually inspected for inconsistencies between retention times across the different samples and signals presenting highly variable retention times (%RSD > 5 %) were discarded. Following this, the most useful *m/z* signals were selected through backward selection via recursive feature elimination, using a random forest model as base learner. R statistical computing software and the *caret* package were used to carry out the latter operation, as described by Kuhn and Johnson [40].

Finally, identities of the compounds associated with the selected *m/z* signals were investigated. For this purpose, a list of possible elemental compositions was first generated for each using the heuristic filtering approach suggested by Kind & Fiehn [41], Seven Golden Rules, and the related homonymous freeware. Accurate neutral masses were inserted in the software based on the assumption that all the observed *m/z* signals corresponded to $[M - H]^-$ adducts (other potential adducts were not taken into consideration in this work). All the elements suggested by the software were allowed (i.e., C, H, N, O, P, S, F, Cl, Br and Si), mass accuracy was set to 5 ppm and isotopic abundance error to 15 %. The empirical formulae generated by this filtering step were then manually entered into the ChemSpider database for structural elucidation. The most probable identities for each *m/z* signal were finally suggested, based on their likelihood to form $[M - H]^-$ adducts in negative-mode ESI and previous knowledge of GSR composition [42-44].

Principal component analysis (PCA) was applied to the different datasets in order to investigate latent patterns within the data. Peak areas were used as input variables. These were initially normalised within-sample by total sum normalisation, in order to correct size effects. Variables with near-zero variance were then removed and those remaining were centred before PCA was performed. For GSR samples, variables were additionally scaled. Data pre-treatment and PCA were carried out using R statistical computing software and available packages.

3. Results and discussion

3.1. Compatibility of EtOH with IC-HRMS

The influence on HRMS signals of eluents based on ethanol (EtOH) was first investigated and compared to other organic modifiers, including acetonitrile (MeCN), methanol (MeOH) and isopropanol (iPrOH). As expected, an EtOH modification enhanced HRMS

signals of all the tested probes in comparison to those observed in a fully aqueous eluent (Fig. 1). This was especially true for signals of the two monovalent anions (i.e., nitrate and perchlorate). Indeed, their intensities were increased by approximately 4 - 5 fold, while intensities of the other anionic probes (which included four deprotonated organic acids and one divalent inorganic anion) were enhanced 2 - 3 fold.

Overall, consistent enhancement effects were observed across the different organic modifiers. It is nonetheless interesting to note that MeCN-based eluents offered the best signals for sulfate and citrate, while iPrOH-based eluents resulted in the best intensities for nitrate and perchlorate. MeOH-based eluents, in particular, yielded the poorest intensities for the latter two ions, with statistically significant differences in comparison to those based on iPrOH. This indicated a more efficient volatilisation of these analytes as a function of the alcohol chain length and alkylation. In addition, the differences in performance between aprotic and protic solvents is likely explained by their influence on hydrogen bonding, especially for compounds such as perchlorate. MeCN, indeed, is likely to disrupt hydration of perchlorate more than protic solvents. Likewise, the opposite was true for sulfate, which is generally less hydrated and thus more affected by MeCN than by protic solvents. In any case, an EtOH-based eluent generally gave intermediary signal enhancements across all tested probes and offered a good compromise.

The anion-exchange column selected here was packed with 5.0 µm particles of a quaternary ammonium-functionalised polyvinyl alcohol polymer and was fully solvent-compatible. Preliminary attempts at using a 50:50 EtOH:H_2O eluent at standard laboratory conditions (20 ℃), however, generated backpressure s above the upper recommended pressure threshold (i.e., 200 bar) even at slow flow rates (0.10 – 0.14 mL min^{-1}). This was not surprising given the viscosity of the mixture (η = 2.4 mPa s^{-1}), which was considerably higher than those of 50:50 MeCN:H_2O, 50:50 MeOH:H_2O and pure water (η = 0.8, 1.5 and 0.9 mPa s^{-1}, respectively). Changes in the backpressure were thus investigated as a func-

tion of the oven temperature (30 - 50 ℃) and flow rate (0.05 - 0.20 mL min^{-1}). An acceptable pressure of ~ 180 bar was observed at 0.18 mL min^{-1} (corresponding to a linear velocity of 0.96 mm s^{-1}) and 50 ℃ (Fig. S1 in ESM), which were thus selected as optimal.

3.2. Optimisation of the IC-HRMS method

The effect of an EtOH-based eluent on the IC separation of the 25 analytes was investigated. Under isocratic conditions, EtOH modification decreased IC retention times compared to a fully aqueous eluent for most analytes at equivalent ionic strengths (Fig. 2). Citrate, perchlorate and thiocyanate were particularly affected and significant selectivity changes were also observed. In comparison to water, EtOH has a lower dieletric constant (ε = 80.1 versus 24.5, respectively). Thus, the general decrease in retention was also likely due to the decrease in eluent polarity and ion hydration radii, as found previously [18]. As expected, reduced SCD intensities for all analytes were also observed.

The void time (t_0) was seen to increase. This indicated shrinkage of the packed polymer bed by ~ 6 %. Despite this, peak symmetry, selectivity and efficiency for analytes with significant tailing in fully aqueous eluents (i.e., benzoate, thiosulfate, citrate and perchlorate) improved with EtOH modification. This was especially true for benzoate, for which the peak showed significant tailing over several minutes in water, but was well resolved and symmetrical in EtOH-based eluents (Fig. 3, peak 10). Benzoate was previously shown to be relevant to the analysis of low-order explosives [15, 37, 45] and this represented a desirable improvement for forensic applications.

Retention was further studied as a function of the eluent ion concentration. Over the tested range, their relationship was linear, with slopes proportional to the charge on the anion (Fig. S2 in ESM). This showed that the retention mechanism was mainly based on anion-exchange, which is not always the case for other solvent systems and/or IC resins, and makes retention behaviour much more difficult to interpret [18]. Following several at-

tempts under isocratic conditions, the best IC separation of all selected anions could only be achieved with gradient elution. Insertion of a linear gradient from 1/0.3 to 15/4.5 mM CO_3^{2-}/HCO_3^- after the elution of nitrate was sufficient to elute all 25 anions within 48 min (Fig. 3a). A larger final concentration of eluting ions was not found to further significantly decrease the total runtime. This was compared with a previous fully optimised gradient IC method using aqueous eluents. Despite the differences in IC selectivity, comparable overall runtimes were obtained (Fig. 3b). Rises in baseline conductivity across both gradients were also similar.

Direct coupling of IC to HRMS was performed. HRMS conditions were optimised by a two-step DOE approach, which involved the evaluation of surface responses (detailed results are provided in ESM). After coupling, a mixed standard of the 25 target anions and the internal standard (IS) was injected and the respective HRMS spectra examined in order to choose adequate quantifier ions and exact *m/z* values. Of the 25 analytes used for the optimisation of the separation step, the *m/z* values for six were beneath the lower threshold of the Orbitrap mass analyser (i.e., *m/z* 50) and did not form any measurable adducts. These were fluoride, chloride, cyanate, azide, formate and nitrite. The remaining 19 anions, on the contrary, could all be detected as the singly deprotonated forms of their respective acids, i.e. $[M - H]^-$ (Fig. 4). As the previously six undetected anions may still be of interest in explosive analysis [5], the use of time-of-flight analysers (minimum *m/z* of 20) or ion association reagents through post-infusion [46, 47] may be considered in future works.

At optimised conditions, natural isotopes for all the detectable anions were observed thanks to the high resolution and mass accuracy allowed by the HRMS instrument, permitting confirmatory detection via relative isotopic abundances (RIAs) ([24]). This was especially true for chlorinated (i.e., chlorite, chlorate, perchlorate) and brominated (i.e., bromide, bromate) species. Figure 5 shows examples of the observed full scan HRMS iso-

topic patterns for selected anions. The most abundant isotope for each target anion was selected as the quantifier (Table 1). Corresponding chromatographic peaks were characterised using 70 – 90 HRMS data points at 100 mg L^{-1}, approximately, demonstrating the possibility of obtaining highly reliable averaged mass spectra even at the lowest concentration ranges.

3.3. Analytical method performance

From Figure 4, IC-HRMS selectivity for the 19 detectable analytes was excellent with a single chromatographic peak observed for each quantifier ion. Benzoate was the only exception, with the presence of a second peak at *m/z* = 121.0295 belonging to the $[M - COOH]^-$ ion of phthalate, whichwas, nevertheless, completely resolved. The elevated discrimination power of the method was particularly evident in the case of sulfate (*m/z* = 96.9601) and phosphate (*m/z* = 96.9696), which partially co-eluted but were still perfectly separated. This would not be possible with low resolution mass analysers. Unlike previous work [18], no background signals were obtained from hydrolysis of the alkaline eluent (e.g. acetate from MeCN hydrolysis in hydroxide); $HS^{18}O_3^-$ and $H^{34}SO_3^-$ from the residual suppressor regenerant were also completely resolved in HRMS from ClO_4^-.

A summary of other method performance characteristics is reported in Table 1. Repeatability of retention times was excellent, with observed %RSDs < 0.4 %. Repeatability of peak areas was satisfactory, with %RSDs generally < 10%. In this regard, %RSDs of chlorite were higher due to instability in solution and the poorest performance was observed for benzoate at 1000 µg L^{-1}. Overall, however, repeatability was better than previous work [18], perhaps due to an improved IC selectivity in gradient elution mode, thereby reducing analyte ion-ion interaction or adduct formation in the gas phase [24]. The same was true for *m/z* accuracies, as all were within the recognised threshold of 5 ppm.

Estimated LODs were excellent and appropriate for practical forensic application. Indeed, these ranged from 0.31 to 7.86 µg L^{-1} (i.e., 6.14 to 157.25 pg on column) for all but four ions. LODs for acetate, oxalate and phosphate were higher but still lay in the low-mid µg L^{-1} range. This was most likely due to a small background occurrence in solvents. The LOD for sulfate was estimated to be > 500 µg L^{-1}, but it was expected since sulfuric acid was used as suppressor regenerant. This can be substituted, if required. Comparison of estimated LODs with a previous IC-HRMS method based on MeCN-based eluents revealed largely comparable results [18], albeit separating almost double the number of targeted ions here. One important consideration underpinning these performances was the configuration of an eluent ion trap between the eluent mixer and the injection valve. Indeed, preliminary tests showed that this was necessary to reduce contamination from heavily retained multi-valent anions, which otherwise significantly compromised sensitivity (data not shown).

Linearity was very good with $R^2 \geq 0.99$ observed for all anions except acetate and sulfate, for which the method was only considered semi-quantitative. Linearity was described either by linear or quadratic least-squares regression equations. Overall, linear ranges were found to be large, with those of some analytes (i.e., tartrate, phthalate and chromate) extending over more than three orders of magnitude. Thiocyanate, bromate, bromide, chlorate and perchlorate seemed to especially benefit from the use of quadratic equations. This was, however, not surprising since curvature in Orbitrap calibrations has been shown to occur frequently in other applications [24, 48].

Lastly, it is noteworthy that no accumulation of Na_2CO_3/$NaHCO_3$ residues in the ESI source was observed during long sequences with the ethanol-enhanced method, contrary to those using aqueous eluents, and this is likely because of an “auto-cleansing” effect in organic solvent. This guaranteed comparable and stable signals over several days. Furthermore, injection of 100 % aqueous extracts into the ethanol-enhanced IC system did

not affect peak shapes, in contrast to what is observed when solvent-containing extracts are injected into purely aqueous IC systems. This represents significant added flexibility for direct analysis of complex sample extracts with less potential for losses due to additional solvent exchange steps.

3.4. Targeted qualitative analysis of Pyrodex residues in sweat

In order to show its improved capability for ionic explosive residues, the method was applied to the detection of trace Pyrodex residues in different kinds of sweat samples. This was therefore an example application to demonstrate proof-of-concept in a pre-blast scenario and potentially prove contact with the intact material. Sweat samples consisted of palm swabs and fingermarks deposited on glass that were collected from a volunteer at different times after handling 2 g of Pyrodex powder for about 2 min, followed by their extraction and direct injection onto IC-HRMS in full scan mode, without the need for dilution or solvent exchange steps that are required under aqueous elution conditions. In line with previous works [15], extracts of the original Pyrodex powder revealed high levels of benzoate, nitrate, chlorate and perchlorate (Table 2). These were likely due to the presence of sodium benzoate (burn rate modifier), potassium nitrate and potassium perchlorate (both oxidisers) in the formulation; presence of chlorate was explained through the degradation of perchlorate, which can occur with perchlorate-based black powder substitutes [19, 49].

Amongst the 19 targeted anions, 13 were confirmed in all samples, including the four anions characteristic of Pyrodex. For the remaining six (i.e., acetate, chlorite, bromate, bromide, phosphate and tartrate), intermittent occurrence was observed, if any. For all the identified compounds, inaccuracies in *m/z* measurements were generally < 3 ppm for all monitored ions, even for those belonging to the least intense isotopes. RIAs met those expected in nature, with absolute deviations usually < 5 %, which was excellent and particularly helpful to support forensic level identification. These chemical characteristics would

be impossible, or very difficult, to measure using a traditional IC-ECD or low resolution IC-MS method. Thus, IC-HRMS offered enhanced identification power, which ultimately could negate further confirmation with additional techniques. The developed method, in particular, had excellent measurement performances over very large concentration ranges, in addition to a more convenient alignment with current sampling/extraction procedures.

3.5. Extension to targeted quantitative analysis and profiling

The concentration of anions as a function of time since handling in palm sweat samples was evaluated next (Table 2). Benzoate, nitrate and perchlorate lay between high µg and mg levels immediately after powder manipulation and showed a clear decrease as a function of time, which was roughly one order of magnitude after 3 h. All amounts reduced to low µg levels after hand washing. The extent of depletion, however, varied between analytes. Benzoate was slightly more affected than nitrate and perchlorate, with both time since handling and hand washing. Amounts of chlorate, as expected, were significantly lower than the others immediately after handling (i.e., µg levels), but they slightly increased after 1 h after handling, which may indicate biodegradation of perchlorate to trace chlorate on hands [19, 49]. After hand washing, concentration of chlorate lay below its LOQ.

Perhaps owing to the improved sensitivity of the method, residual anionic content in samples (included those related to Pyrodex) was also identified in negative controls at very low concentrations. Statistical comparison showed that anions determined in sweat/fingermark samples were significantly higher than this residual level ($p < 0.05$ for all the cases except nitrate and perchlorate in fingermarks, for which $p < 0.1$ applied), thus supporting the reliability of the approach to establish contact with Pyrodex.

The quantity of each ion determined obviously depended on the quantity recovered, which is often highly variable [50]. Therefore, evaluation using single ions was considered a poor approach to infer Pyrodex handling. Using a multi-variate approach, principal com-

ponent analysis (PCA) was applied here as an alternative. Score plots obtained after PCA performed on the four Pyrodex-related analytes showed excellent discrimination between positive and negative samples (Fig. 6a-b). Moreover, low concentrations of eight other ions were detectable in samples, which likely related to the sweat and/or external contamination on the hands or the surface. PCA applied to the full set of ions (Fig. 6c-d) revealed a separation between positive and negative samples, but, most importantly, now positive samples could be additionally discriminated by their time since handling in PC1. Loadings showed that samples taken immediately after Pyrodex handling were characterised by particularly high proportions of perchlorate and nitrate. Negative sweat and fingermark samples, on the contrary, were characterised by high proportions of lactate and sulfate, where lactate was likely to arise endogenously. This supplementary information is potentially very useful in forensic cases and moves beyond the scope of traditional targeted explosives detection approaches. Here, the advantage of a more comprehensive, full scan IC-HRMS profiling approach was therefore clear in comparison to most traditional IC or IC-MS based methods, which are not usually applied to complex matrices in a quantitative manner.

3.6. Targeted and non-targeted discrimination of GSRs from different sources

Pyrodex is arguably a rather simple mixture. So, the above approach was extended to classification of different ammunition types based on their GSRs, which have previously been shown to display much more complex anionic profiles [15, 37]. This also represented a post-discharge scenario, where the ingredients of the ammunition are unlikely to match the associated GSR in terms of relative abundance. Targeted analysis identified 15 anions across the different ammunition types. Phosphate and tartrate were never detected. The same was true for chlorite and chlorate, even though perchlorate was detected in the GSRs of two ammunition types. Figure 7a-b shows the score plots after PCA for extracted peak areas of the 15 compounds only. GSR samples from Geco formed a cluster that was

significantly removed from the those formed by the other two ammunition types. Examination of loadings showed that these were characterised by particularly high proportions of threonate, benzoate, bromide, nitrate, oxalate and thiocyanate. A good separation was also observed for negative controls, which, on the contrary, were characterised by high proportions of acetate, sulfate, phthalate and citrate. GSR samples from Federal and Remington, however, formed clusters in close proximity, which suggested similar compositions for these 15 targeted anions. Discrimination between them was thus more challenging based on targeted analysis alone.

In order to utilise the power of IC-HRMS more comprehensively, full scan data were further examined using a non-targeted approach. Investigation of *m/z* ion maps highlighted the presence of a number of additional peaks not corresponding to the original targeted anions (data not shown). Some had relatively high *m/z* values, above that of citrate. In order to use this additional chemical information for GSR classification, automated peak picking was performed. The procedure shortlisted 79 new *m/z* signals that were common amongst different ammunition types. These were further filtered through backward selection via recursive feature elimination in order to retain a set of 20 potentially characteristic analytes, with *m/z* values ranging from 69.0207 to 421.2270. A total of 14 corresponded to ions with $m/z > 100$. This highlights a potential further advantage of using ethanolic eluents, as the resultant disruption of non-polar interactions with the IC resin affects elution of more, potentially discriminating anions, which may not elute at all under aqueous conditions. Formal identification of these analytes was beyond the scope of this work, but a list of preliminary candidates can be found in Table 3. Most of them were in agreement with compounds previously identified in GSR using other analytical techniques [42-44], but some were newly reported, as for example methylbenzoate and phenylpropanoate.

Application of PCA (Fig. 7c-d) to the peak areas revealed that, contrary to the previous situation, GSR samples from the three different ammunition types and negative con-

trols now formed completely separated clusters in score plots. In particular, the slight overlap between Federal and Remington observed with targeted-analysis PCA was now totally resolved using the 20 new characteristic signals. Examination of loadings showed that GSR samples from Federal ammunition were characterised by particularly high signals at *m/z* 101.9608, 115.9765, 135.0453, 143.0609, 149.0609, 152.9864, 152.9977, 199.1704, 227.2019, 421.2270, Remington by high signals at *m/z* 84.0204, 69.0207, 112.0266, and Geco by high signals at *m/z* 221.0821. PCA was also attempted on a merged dataset of 35 targeted and remaining non-targeted *m/z* signals (Fig. 7e-f). An improved discrimination between all the groups was again observed. Consequently, the new selected *m/z* signals, which were retrospectively obtained thanks to the full scan HRMS data, actually provided additional useful information for separation of GSR samples according to their sources. The developed IC-HRMS method could thus potentially be very helpful also in GSR profiling, for example by integration in recently suggested *in silico* profiling approaches [51].

4. Conclusion

The successful development, validation and application of a gradient ethanol-enhanced IC-HRMS method for targeted and non-targeted analysis of ionic energetic material residues in complex samples were presented here for the first time. In particular, this method offered both excellent qualitative and quantitative capabilities across several orders of magnitude and demonstrated excellent potential for retrospective use and identification of additional ions via full scan HRMS data capture. The incorporation of an ethanolic eluent in IC-HRMS offered five distinct major advantages over traditional aqueous-based IC-MS eluents:

a) it enabled direct integration with extraction procedures currently in use for routine organic energetic materials analysis without requiring solvent exchange or further sample preparation for compatibility with IC;
b) it resulted in excellent reproducibility, likely arising from an inherent 'self-cleaning' effect of both the IC and ESI sources, thereby minimising matrix and/or eluent residue contamination;
c) it altered non-polar/polar and ionic interactions within the ion exchanger and its polymer backbone, as well as in the eluent itself and conferred altered selectivity, facilitated faster elution and permitted detection of several new characteristic, higher molecular weight and forensically useful ions;
d) it simplified traditional IC-MS configurations by rendering additional pumps for post-suppressor solvent infusion unnecessary; and
e) it resulted in more efficient ESI and, as a result, a several fold improvement in detector sensitivity to inorganic and organic anions at the low-mid pg level.

Suitability of the method for forensic applications was successfully tested on fingermarks and hand sweat samples from a volunteer who previously handled a black-powder substitute, as well as gunshot residues from different ammunition types. Detection of all the anions targeted by the method was easily accomplished using selected analyte ion signals. Furthermore, significant discriminatory power was possible between positive and negative samples, as well as the different ammunitions, through both targeted and non-targeted analysis based on the wider use of full scan IC-HRMS data combined with PCA. The success of this IC-HRMS approach therefore places it beyond current IC and IC-MS capability in forensic science and has significant potential in wider applications requiring determination of similar ionic species (e.g., metabolomics and environmental analysis).

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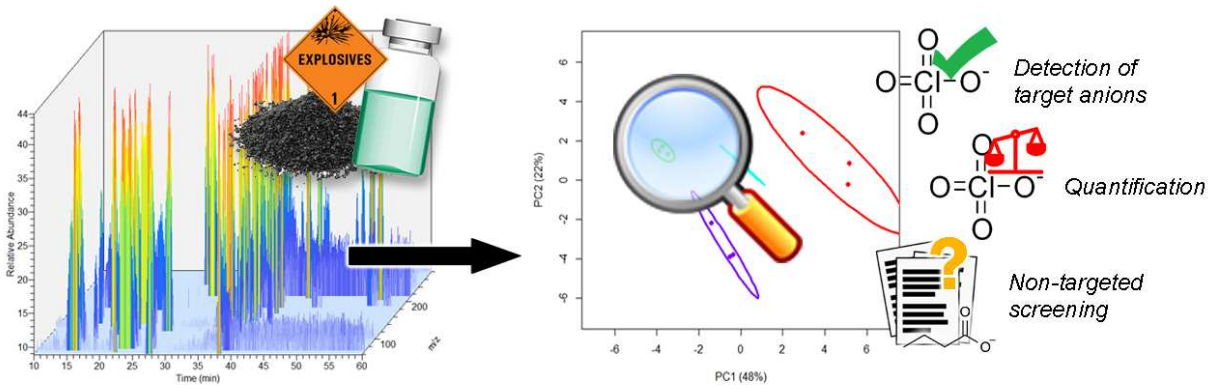

EtOH-enhanced IC-HRMS analysis

Data analysis and mining

TOC image

Table 1 – Figures of merit for the developed ethanol-enhanced IC-HRMS method. Determination ranges were inspected using linear and quadratic models, and are reported as the intervals of concentrations that allow for $R^2 > 0.99$. Inaccuracy of *m/z* value ($\delta_{m/z}$), repeatability of retention time (t_R) and repeatability of peak area (PA) were measured on 6 replicates at two different concentrations: 100 and 1000 µg L^{-1}. Six compounds could not be detected with the HRMS instrument used and only their IC-SCD retention times and exact *m/z* values are reported.

Anion	t_R[a]	Exact m/z[b]	$\delta_{m/z}$ (ppm)		Repeatability t_R (%RSD)		Repeatability PA (%RSD)		LOD		Ref. LOD[c]		LOQ		Upper determination limit (µg L^{-1})	
			@ 100 µg L^{-1}	@ 1000 µg L^{-1}	@ 100 µg L^{-1}	@ 1000 µg L^{-1}	@ 100 µg L^{-1}	@ 1000 µg L^{-1}	µg L^{-1}	pg	µg L^{-1}	pg	µg L^{-1}	pg	Linear	Quadratic
Threonate	12.67	135.0299	0.49 ± 0.38	0.49 ± 0.38	0.21	0.15	2.78	6.01	0.79	15.77	N/A	N/A	2.63	52.55	1,000	5,000
Lactate	12.70	89.0244	0.37 ± 0.58	0.00 ± 0.00	0.24	0.27	13.80	7.69	2.06	41.18	6.00	120.00	6.86	137.25	1,000	5,000
Acetate	12.85	59.0139	1.13 ± 0.88	1.69 ± 0.00	0.33	0.39	8.05	7.69	38.12	762.49	N/A	N/A	127.08	2,541.62	N/A	N/A
Chlorite	14.08	66.9592	0.25 ± 0.61	0.00 ± 0.00	0.22	0.20	14.49	10.33	1.76	35.14	N/A	N/A	5.86	117.14	1,000	5,000
Bromate	16.23	126.9036	0.53 ± 0.41	0.00 ± 0.00	0.19	0.23	3.32	5.11	1.00	19.98	2.85	57.00	3.33	66.60	500	5,000
Benzoate	16.68	121.0295	0.28 ± 0.43	0.69 ± 0.34	0.31	0.30	9.81	18.83	1.82	36.37	3.75	75.00	6.06	121.22	1,000	5,000
Chlorate	19.46	82.9541	0.40 ± 0.62	0.00 ± 0.00	0.13	0.19	3.67	1.98	0.85	17.06	0.53	10.50	2.84	56.86	500	5,000
Bromide	19.69	78.9189	0.42 ± 0.65	1.27 ± 0.00	0.11	0.28	1.46	5.44	1.09	21.81	0.78	15.57	3.63	72.70	500	5,000
Nitrate	19.72	61.9884	0.54 ± 0.83	1.34 ± 0.66	0.14	0.17	4.08	6.51	7.86	157.25	6.00	120.00	26.21	524.16	1,000	5,000
Thiocyanate	26.92	57.9757	0.29 ± 0.70	0.29 ± 0.70	0.14	0.14	4.19	1.74	0.31	6.14	0.61	12.15	1.02	20.48	500	5,000
Perchlorate	31.31	98.9491	0.67 ± 0.52	1.01 ± 0.00	0.19	0.09	4.04	2.80	0.57	11.50	0.12	2.46	1.92	38.32	500	5,000
Phosphate	35.42	96.9696	< LOD	2.06 ± 0.00	< LOD	0.07	< LOD	4.53	127.07	2,541.48	N/A	N/A	423.58	8,471.60	5,000	5,000
Tartrate	36.55	149.0092	0.56 ± 0.51	0.67 ± 0.00	0.13	0.08	4.24	2.80	1.32	26.37	N/A	N/A	4.40	87.91	5,000	5,000
Sulfate	37.77	96.9601	< LOD	0.86 ± 0.42	< LOD	0.11	< LOD	13.21	> 500	> 10,000	N/A	N/A	> 500	> 10,000	N/A	N/A
Oxalate	38.25	88.9880	0.75 ± 0.58	0.00 ± 0.00	0.15	0.06	7.81	4.03	73.07	1,461.35	73.50	1470.00	243.56	4,871.16	5,000	5,000
Phthalate	39.03	165.0193	0.40 ± 0.31	0.40 ± 0.31	0.12	0.09	6.23	2.57	5.85	117.02	4.35	87.00	19.50	390.08	5,000	5,000
Thiosulfate	42.79	112.9373	0.15 ± 0.36	0.74 ± 0.36	0.11	0.06	4.59	4.73	1.79	35.88	N/A	N/A	5.98	119.60	1,000	5,000
Chromate	44.53	116.9285	0.14 ± 0.35	0.14 ± 0.35	0.08	0.05	8.17	5.07	3.74	74.71	N/A	N/A	12.45	249.04	5,000	5,000
Citrate	46.63	191.0197	0.17 ± 0.27	0.17 ± 0.27	0.10	0.07	7.76	8.14	2.57	51.39	N/A	N/A	8.57	171.32	1,000	5,000
Nitrate ^{15}N (IS)	*19.72*	62.9854	0.53 ± 0.82	1.32 ± 0.65	0.09	0.10	2.72	6.56	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fluoride	*12.90*	18.9990	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Formate	*13.46*	44.9982	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cyanate	*16.53*	41.9985	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chloride	*16.56*	34.9694	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nitrite	*16.96*	45.9935	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Azide	*18.46*	42.0098	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected in the final IC-HRMS method (*m/z* < 50) but in IC-SCD.

[a] values determined on the samples spiked at 100 µg L^{-1} (n = 6).

[b] most abundant isotope.

[c] reported by Gilchrist et al. [15].

Table 2 – Results from targeted analysis of perchlorate, benzoate, nitrate and chlorate in Pyrodex-contaminated palm sweat samples and fingermarks (n = 3). The column "Target ion" reports the data concerning the most abundant isotopes of the respective analyte target ion. Data includes inaccuracy in measured *m/z* ($\delta_{m/z}$) and absolute deviation of relative isotope abundance (D_{RIA}), which were interpreted with respect to the intensity of the monoisotopic peak (I_A). Observed retention times (t_R) and estimated amounts are also reported.

Anion (isotopes) / sample	*t_R (min)*	*I_A (counts)*	*Target ion*						*Amount (µg)*[a]
			Most abundant isotope		*2nd isotope*		*3rd isotope*		
			$\delta_{m/z}$ (ppm)	*D_{RIA} (%)*	*$\delta_{m/z}$ (ppm)*	*D_{RIA} (%)*	*$\delta_{m/z}$ (ppm)*	*D_{RIA} (%)*	
Benzoate (A, A+1, A+2)									
Palm, t = 0 h, no washing	16.97 ± 0.06	7E+7 ± 3E+6	0.00 ± 0.00	0.00 ± 0.00	0. 82 ± 0.00	0.25 ± 0.02	0.00 ± 0.00	0.12 ± 0.00	887.9 ± 44.1 (**)
Palm, t = 1 h, no washing	17.08 ± 0.01	3E+7 ± 7E+5	0.00 ± 0.00	0.00 ± 0.00	0. 27 ± 0.47	0.66 ± 0.48	0.27 ± 0.47	0.14 ± 0.01	441.2 ± 12.1 (**)
Palm, t = 3 h, no washing	17.17 ± 0.06	8E+6 ± 9E+5	1.38 ± 0.48	0.00 ± 0.00	0. 82 ± 0.00	1.02 ± 0.44	1.63 ± 0.00	0.23 ± 0.01	90.08 ± 13.14 (**)
Palm, t = 0 h, washing	17.64 ± 0.03	7E+6 ± 4E+6	0.28 ± 0.48	0.00 ± 0.00	0. 27 ± 0.47	2.98 ± 1.91	1.08 ± 0.47	0.33 ± 0.05	2.06 ± 0.06 (**)
Palm, t = 1 h, washing	17.72 ± 0.04	6E+6 ± 2E+5	0.55 ± 0.48	0.00 ± 0.00	0. 55 ± 0.47	1.00 ± 0.27	1.08 ± 0.47	0.27 ± 0.02	2.73 ± 0.10 (**)
Fingermarks, t = 0 h	16.77 ± 0.07	5E+6 ± 2E+6	0.28 ± 0.48	0.00 ± 0.00	0. 00 ± 0.00	1.74 ± 0.61	1.08 ± 0.47	0.25 ± 0.03	N/A (**)
Fingermarks, t = 1 h	16.78 ± 0.07	1E+6 ± 4E+5	1.10 ± 0.48	0.00 ± 0.00	0. 82 ± 0.00	3.07 ± 0.11	2.44 ± 0.81	0.36 ± 0.04	N/A (**)
Chlorate (A, A+2, A+4)									
Palm, t = 0 h, no washing	19.22 ± 0.05	2E+6 ± 1E+5	0.00 ± 0.00	0.00 ± 0.00	1. 18 ± 0.00	4.82 ± 0.44	n.d.	0.20 ± 0.00	1.82 ± 0.05 (**)
Palm, t = 1 h, no washing	19.34 ± 0.02	3E+6 ± 2E+5	0.40 ± 0.70	0.00 ± 0.00	0. 78 ± 0.68	4.44 ± 0.39	n.d.	0.20 ± 0.00	2.79 ± 0.15 (**)
Palm, t = 3 h, no washing	19.39 ± 0.03	6E+5 ± 5E+4	2.41 ± 0.00	0.00 ± 0.00	0. 78 ± 0.68	4.51 ± 2.39	n.d.	0.20 ± 0.00	0.38 ± 0.03 (**)
Palm, t = 0 h, washing	19.49 ± 0.03	3E+5 ± 4E+4	1.21 ± 0.00	0.00 ± 0.00	0. 00 ± 0.00	4.04 ± 0.57	n.d.	0.20 ± 0.00	<LOQ (**)
Palm, t = 1 h, washing	19.51 ± 0.03	3E+5 ± 4E+4	1.21 ± 0.00	0.00 ± 0.00	0. 39 ± 0.68	4.31 ± 2.27	n.d.	0.20 ± 0.00	<LOQ (**)
Fingermarks, t = 0 h	19.42 ± 0.03	2E+5 ± 1E+5	0.80 ± 0.70	0.00 ± 0.00	0. 39 ± 0.68	9.21 ± 3.65	n.d.	0.20 ± 0.00	N/A (**)
Fingermarks, t = 1 h	19.41 ± 0.03	1E+5 ± 8E+4	1.81 ± 0.85	0.00 ± 0.00	0. 59 ± 0.83	7.65 ± 2.17	n.d.	0.20 ± 0.00	N/A (**)
Nitrate (A, A+2)									
Palm, t = 0 h, no washing	19.69 ± 0.06	5E+8 ± 2E+7	1.61 ± 0.00	0.00 ± 0.00	1. 56 ± 0.00	0.01 ± 0.00	N/A	N/A	2212 ± 70 (**)
Palm, t = 1 h, no washing	19.72 ± 0.00	4E+8 ± 9E+6	1.61 ± 0.00	0.00 ± 0.00	1. 56 ± 0.00	0.00 ± 0.00	N/A	N/A	1559 ± 54 (**)
Palm, t = 3 h, no washing	19.72 ± 0.00	2E+8 ± 2E+7	0.00 ± 0.00	0.00 ± 0.00	0. 00 ± 0.00	0.04 ± 0.00	N/A	N/A	890.4 ± 85.0 (**)
Palm, t = 0 h, washing	19.72 ± 0.01	1E+8 ± 1E+7	0.00 ± 0.00	0.00 ± 0.00	0. 52 ± 0.90	0.09 ± 0.01	N/A	N/A	26.82 ± 5.09 (**)
Palm, t = 1 h, washing	19.74 ± 0.02	2E+8 ± 1E+7	0.00 ± 0.00	0.00 ± 0.00	0. 00 ± 0.00	0.05 ± 0.01	N/A	N/A	56.07 ± 4.72 (**)
Fingermarks, t = 0 h	19.75 ± 0.06	2E+8 ± 2E+8	1.08 ± 0.93	0.00 ± 0.00	1. 56 ± 0.00	0.14 ± 0.20	N/A	N/A	N/A (*)
Fingermarks, t = 1 h	19.73 ± 0.06	7E+7 ± 3E+7	0.00 ± 0.00	0.00 ± 0.00	0. 00 ± 0.00	0.05 ± 0.03	N/A	N/A	N/A (*)
Perchlorate (A, A+2, A+4)									
Palm, t = 0 h, no washing	31.24 ± 0.06	5E+8 ± 2E+7	1.01 ± 0.00	0.00 ± 0.00	1. 98 ± 0.00	1.54 ± 0.06	n.d.	0.26 ± 0.00	1099 ± 38 (**)
Palm, t = 1 h, no washing	31.33 ± 0.01	4E+8 ± 1E+7	0.67 ± 0.58	0.00 ± 0.00	0. 99 ± 0.00	1.48 ± 0.09	n.d.	0.26 ± 0.00	839.8 ± 25.2 (**)
Palm, t = 3 h, no washing	31.34 ± 0.02	2E+8 ± 2E+7	0.34 ± 0.58	0.00 ± 0.00	0. 00 ± 0.00	1.55 ± 0.04	n.d.	0.26 ± 0.00	413.2 ± 52.5 (**)
Palm, t = 0 h, washing	31.32 ± 0.02	1E+8 ± 2E+7	0.00 ± 0.00	0.00 ± 0.00	0. 33 ± 0.57	1.21 ± 0.11	n.d.	0.26 ± 0.00	20.13 ± 3.82 (**)

Palm, t = 1 h, washing	31.31 ± 0.02	2E+8 ± 2E+7	0.34 ± 0.58	0.00 ± 0.00	0. 33 ± 0.57	1.45 ± 0.15	n.d.	0.26 ± 0.00	33.11 ± 3.66 (**)
Fingermarks, t = 0 h	30.23 ± 0.02	9E+7 ± 4E+7	0.34 ± 0.58	0.00 ± 0.00	0. 00 ± 0.00	1.12 ± 0.08	n.d.	0.26 ± 0.00	N/A (*)
Fingermarks, t = 1 h	30.21 ± 0.02	5E+7 ± 3E+7	0.67 ± 0.58	0.00 ± 0.00	0. 99 ± 0.00	1.06 ± 0.25	n.d.	0.26 ± 0.00	N/A (*)

"n.d.": not detected; "< LOQ": analyte detected but concentration below its lower limit of quantitation.

[a] (**) and (*) means that observed PA signals were statistically different from those observed in blanks, at 0.05 and 0.1 significance levels, respectively.

Table 3 – List of new *m/z* signals detected in GSR samples after non-targeted analysis, with related retention times (t_R) and most probable identities suggested after preliminary structural elucidation. "Potential formulae" refers to the number of possible elemental compositions obtained after heuristic filtering using Seven Golden Rules, whilst inaccuracy of *m/z* values ($\delta_{m/z}$) is calculated as the difference between the measured *m/z* and that of the most probable compound identified for that signal.

#	Measured m/z	t_R (min)	Isotopic pattern (%)[a]			Potential for-mulae	Suggested identity		
			A+1	A+2	A+3		Formula	Name	$\delta_{m/z}$ (ppm)
1	69.0207	16.11 ± 0.14	0.77	0.00	0.00	0	N/A	N/A	N/A
2	80.9651	14.12 ± 0.18	0.10	0.00	0.00	0	N/A	N/A	N/A
3	84.0204	16.28 ± 0.16	1.11	0.05	0.00	1	$C_2H_2N_3O^-$	N'-Cyanocarbamimidate	-1.19
4	87.0452	12.76 ± 0.18	1.81	0.00	0.00	1	$C_4H_7O_2^-$	Butyrate	-1.15
5	96.9585	44.39 ± 0.86	0.00	0.00	0.00	0	N/A	N/A	N/A
6	96.9761	12.63 ± 0.15	2.83	0.00	0.00	0	N/A	N/A	N/A
7	101.0608	12.89 ± 0.22	2.84	0.02	0.00	1	$C_5H_9O_2^-$	Valerate	0.00
8	112.0266	17.14 ± 0.17	0.92	0.00	0.00	0	N/A	N/A	N/A
9	115.0765	37.02 ± 0.55	4.34	0.00	0.00	1	$C_6H_{11}O_2^-$	Capronate	-0.87
10	135.0453	16.73 ± 0.24	7.33	0.00	0.00	2	$C_8H_7O_2^-$	3-Methylbenzoate	-1.48
11	143.1078	17.04 ± 0.14	6.34	0.00	0.00	1	$C_8H_{15}O_2^-$	Caprylate	0.00
12	149.0609	16.81 ± 0.21	6.58	0.00	0.00	2	$C_9H_9O_2^-$	3-Phenylpropanoate	-0.67
13	152.9864	37.89 ± 0.11	1.52	0.00	0.00	2	N/A	N/A	N/A
14	152.9977	14.69 ± 0.22	1.03	0.00	0.00	5	N/A	N/A	N/A
15	199.1704	13.58 ± 0.15	9.16	0.00	0.00	1	$C_{12}H_{23}O_2^-$	3-Hydroxy-dodecan-1-one	-0.50
16	221.0821	14.56 ± 0.21	9.4	0.39	0.00	10	$C_{12}H_{13}O_4^-$	4-Pentanoyloxybenzoate	-0.90
17	227.2019	13.68 ± 0.22	10.44	0.00	0.00	1	$C_{14}H_{27}O_2^-$	Myristate	-0.88
18	281.2486	14.03 ± 0.21	14.36	0.49	0.00	1	$C_{18}H_{33}O_2^-$	Petroselinate	0.00
19	293.1760	12.68 ± 0.27	12.29	0.00	0.00	15	$C_{17}H_{25}O_4$-	3,4-Dipentoxybenzoate	-0.68
20	421.2270	14.55 ± 0.15	17.65	1.24	0.04	179	$C_{21}H_{30}FN_4O_4^-$	2-[1-(2-Fluoro-4-methoxybenzyl)-3-oxo-2-piperazinyl]-N-[3-(4-morpholinyl)propyl]acetamide	-3.09

[a] percentage of the intensity compared to that of the respective monoisotopic peak (A).

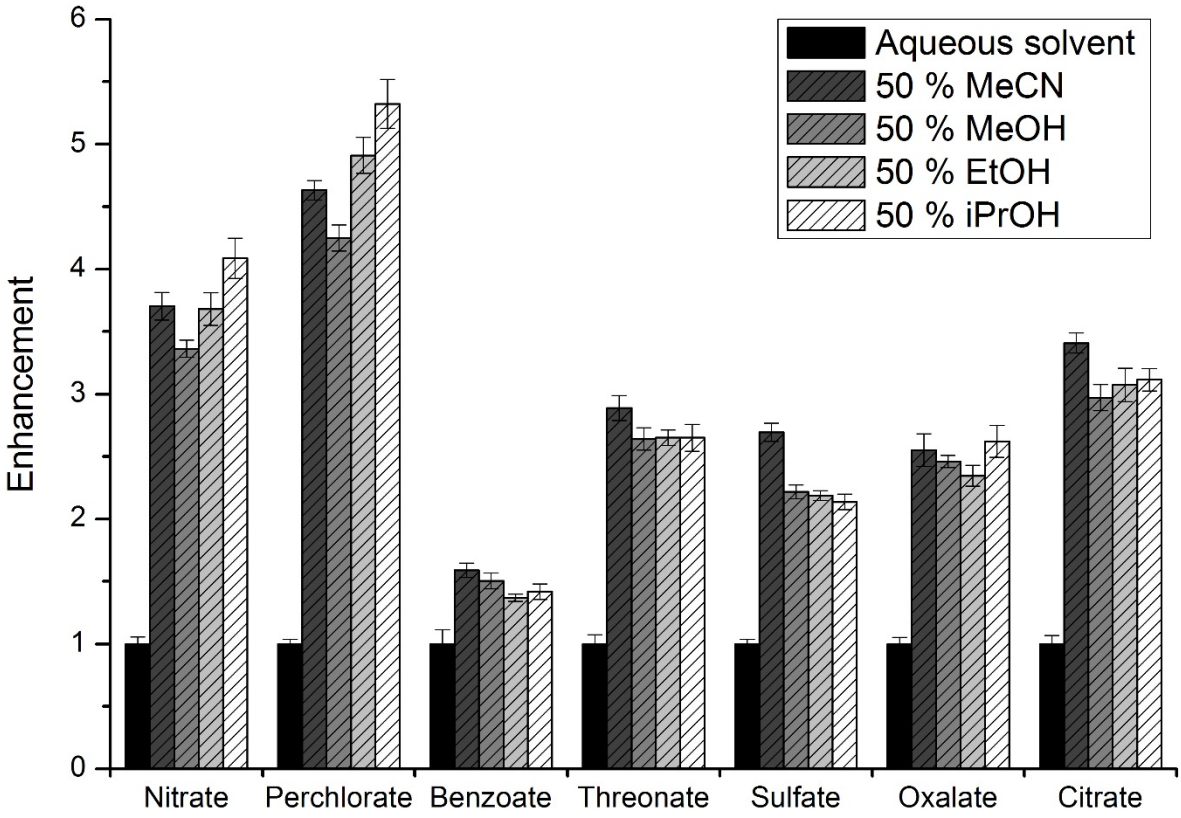

Figure 1 – Comparison of the enhancement effects on the HRMS signals of 7 anionic probes, seen as a result of the modification of a fully aqueous eluent with 50% of four different organic solvents. Tests were performed by direct infusion.

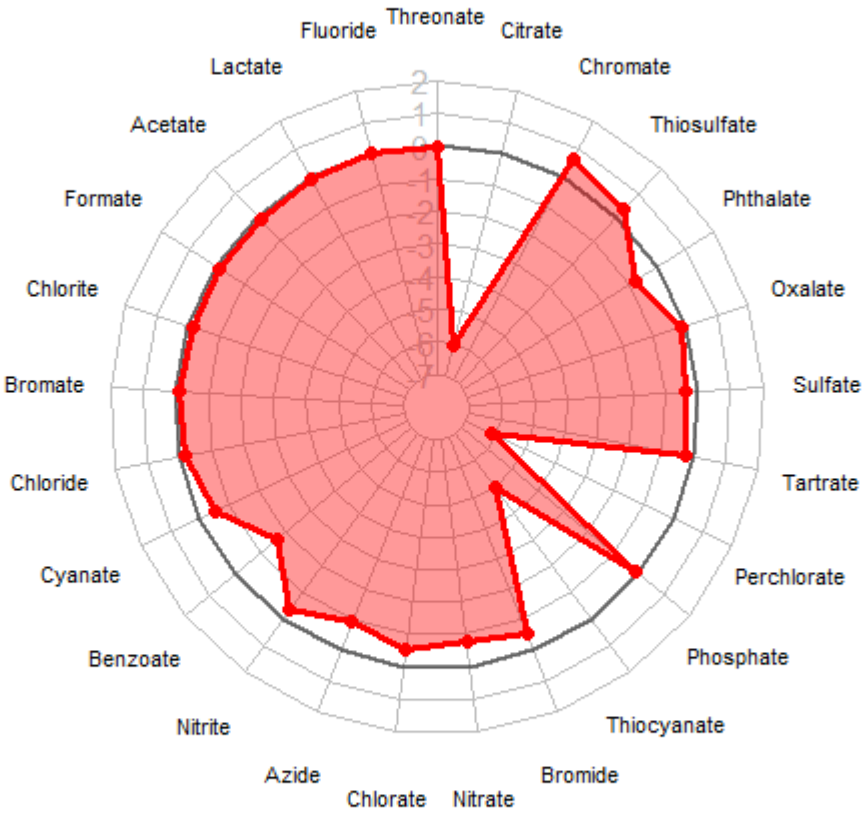

Figure 2 – Radar plot showing the differences between the retention factors (Δk) of the 25 target anions observed in a 50:50 EtOH:H_2O and a fully aqueous eluent at a strength of 5/1.5 mM CO_3^{2-}/HCO_3^- (isocratic conditions). Δk were calculated by k_{EtOH} - k_{H2O}. Negative values indicate a decrease of k (and retention time) in 50:50 EtOH:H_2O compare to full water.

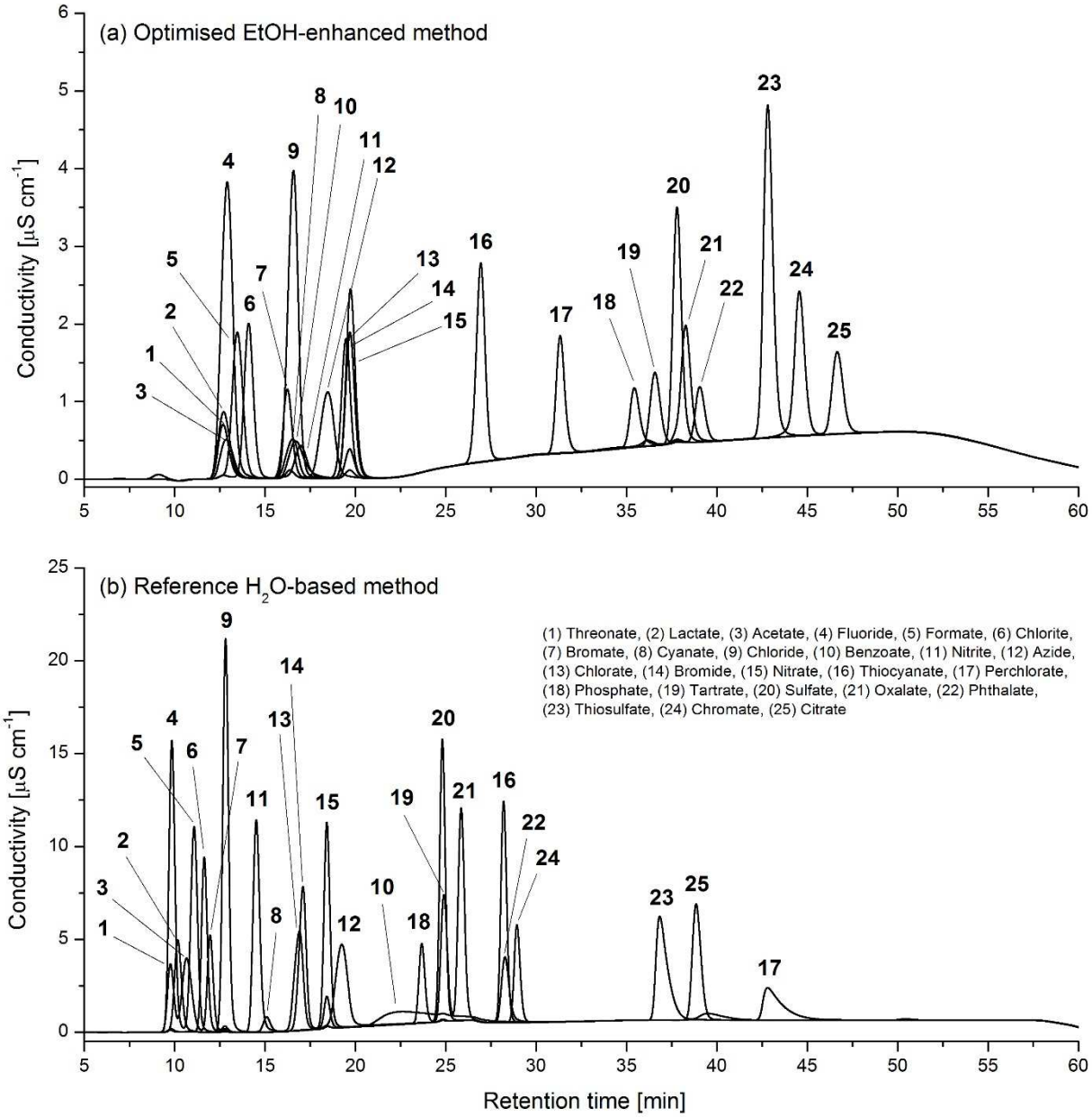

Figure 3 – Comparison of IC-SCD chromatograms between (a) the ethanol-enhanced method optimised in this work and (b) a previous fully aqueous method optimised using the same instrument and column.

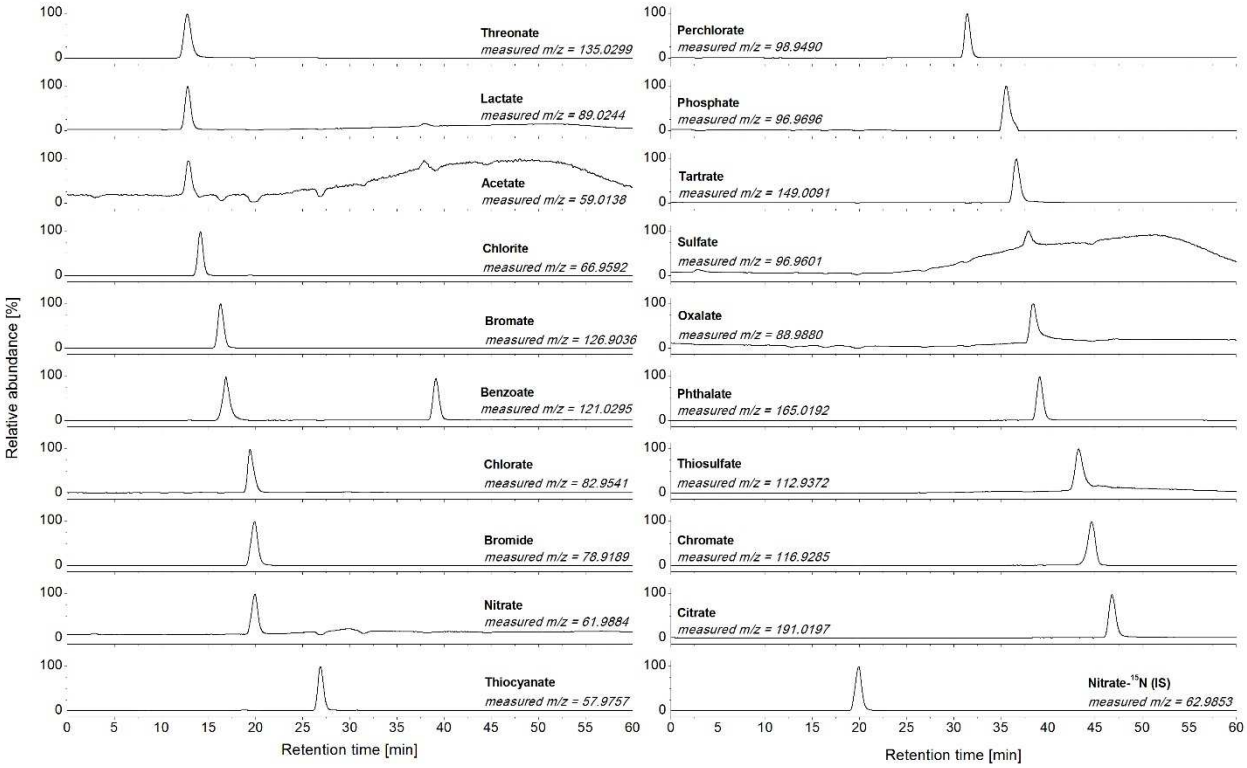

Figure 4 – Extracted ion chromatograms (EICs) of a mixed solution of the 19 target anions plus the internal standard (IS) at 1 mg L^{-1} in 50:50 EtOH:H_2O, after analysis by the developed IC-HRMS method

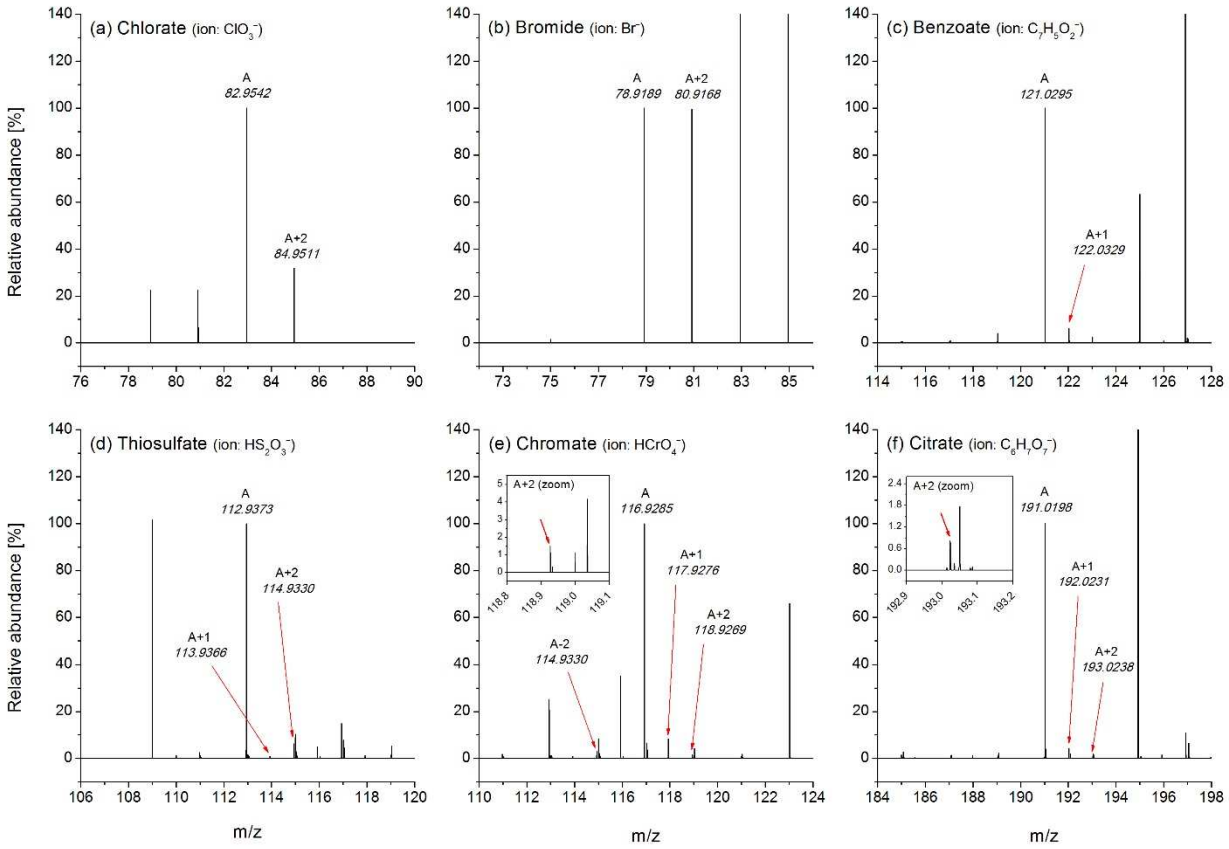

Figure 5 – Isotope patterns for six targeted anions observed after analysis with the developed IC-HRMS method. A perfect resolution of *m/z* signals belonging to some minor isotopes in the presence of other isobaric compounds due to unidentified contaminations can be noticed (insets). "A" refers to the monoisotopic peaks.

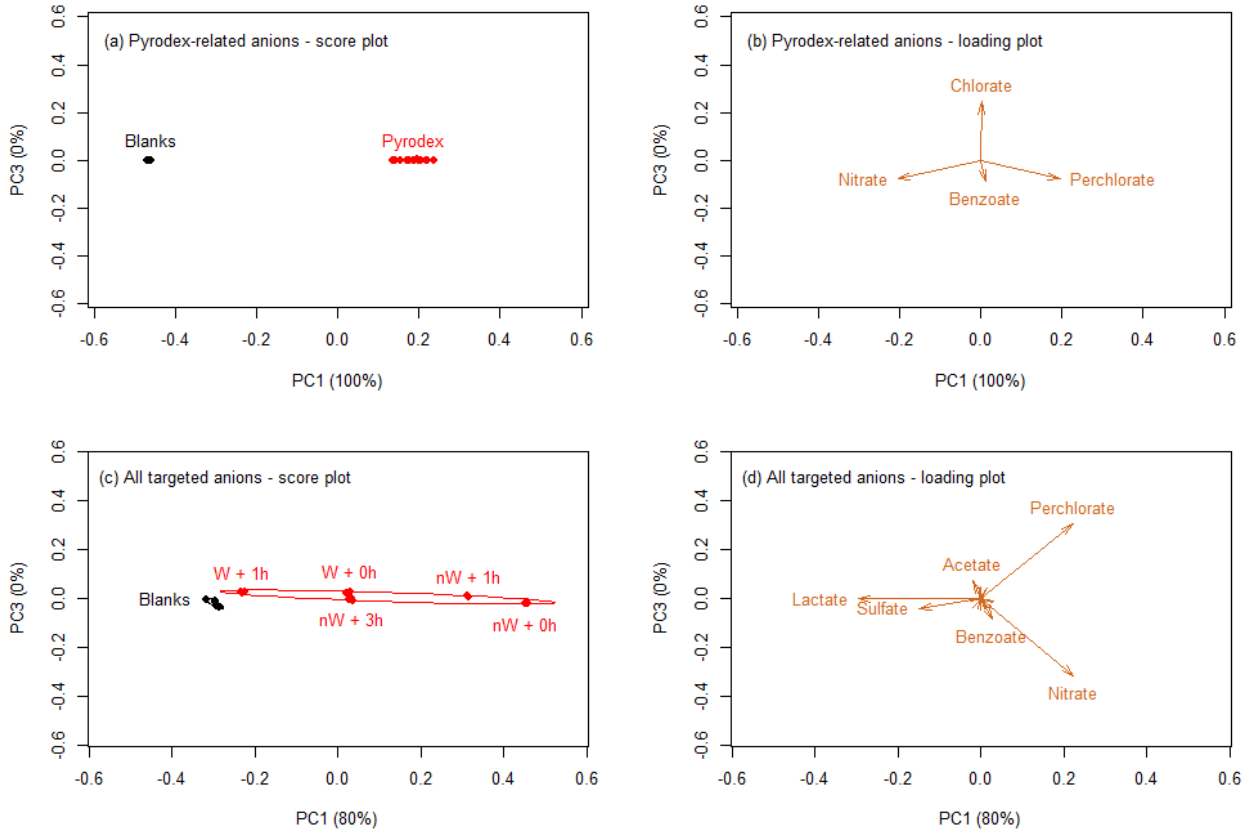

Figure 6 – Score and loading plots after principal component analysis (PCA) of the data acquired from the IC-HRMS analysis of hand-swab extracts that were collected from a volunteer after Pyrodex handling. PCA has been carried out on the extracted peak areas of (a-b) the only four Pyrodex-related anions and (c-d) all the anions targeted with the developed method. "W" and "nW", respectively, indicate if hands were washed or not with tap water immediately after Pyrodex handling; times indicate the periods of time elapsed between handling and swab sampling.

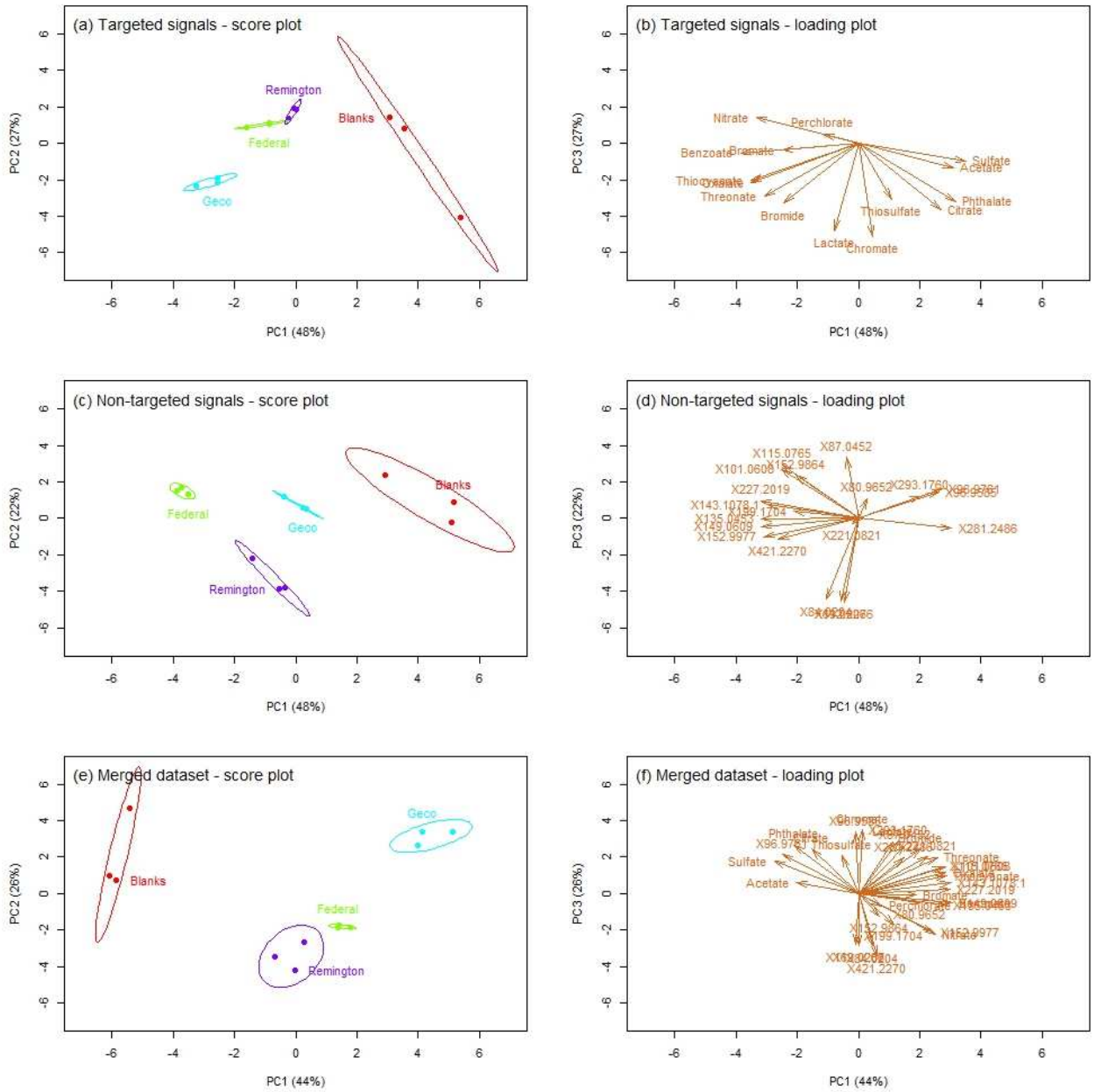

Figure 7 – Score and loading plots after principal component analysis (PCA) of the data acquired from the IC-HRMS analysis of GSRs extracted from the spent cases of different ammunition types. PCA has been carried out on (a-b) all the signals initially targeted by the developed method, (c-d) a series of signals selected after non-targeted analysis of IC-HRMS chromatograms and (e-f) the two merged datasets.

Highlights

- A novel gradient IC-HRMS approach developed and validated for explosive analysis.
- An ethanolic eluent allowed direct analysis of extracts in organic solvent.
- Both quantitative targeted and non-targeted analysis demonstrated.
- Retrospective HRMS data mining approach applied for advanced forensic applications.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: